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STUDIES OF INSECTS AFFECTING THE PRODUCTION OF RED CLOVER SEED

BY

FAUSTINO QUESALES OTANES
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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Faustino Quesales Otanes

ENTITLED Studies of Insects Affecting the Production of Red Clover Seed.

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science

J. W. Folsom
In Charge of Thesis

E. L. Metcalf
Head of Department

Recommendation concurred in*

Committee on Final Examination*

*Required for doctor's degree but not for master's

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STUDIES OF INSECTS AFFECTING THE PRODUCTION OF RED CLOVER SEED

I. INTRODUCTION

With soil and weather conditions all favorable to the growth and flowering of red clover, a low yield of seed can be attributed chiefly to two factors, namely, the absence or scarcity of insect pollinators and the abundance of insects that affect the seeds directly. The most important insects that infest the heads of clover are the clover seed-midge (Dasyneura leguminicola Lint.), the clover seed-chalcid (Bruchophagus funebris How.), and the clover seed-caterpillar (Enarmonia interstinctana Clem.). It is the object of this paper to present the observations that were made on these pests during the spring and summer of 1921 in the hope that they will be of some value to workers engaged in the control, or in the study of the control, of these clover pests. The points especially dealt with in this paper are the periodic abundance of these insects and the relation of such abundance to the production of red clover seed, and the control of insects by means of pasturing, clipping, or early cutting of the June hay crop.

With a limited time at his disposal, there has been no attempt on the part of the writer to cover every phase of the subject. For example, he has not gone into a detailed study of the life histories and descriptions of the pests dealt with, as the results of accurate investigations that have been published on these subjects by previous workers, both in and outside the state of Illinois, have made such a study unnecessary. Anyone interested in the most important insect

pests of red clover, particularly the three mentioned here, will find a detailed and accurate account of them, especially as regards life history, descriptions and control, in Bulletin 134, by Dr. J. W. Folsom, published in 1909 by the University of Illinois Agricultural Experiment Station. (See also 1909 Report of the State Entomologist of Illinois for the same article by Dr. Folsom.)

In the present paper an account of observations made by the writer on the lesser clover leaf-weevil (Phytonomus nigrirostris Fab.) is included, however, since so far as the writer knows, no data have been published in regard to the occurrence and work of this insect in Illinois. There are also included in this paper some observations on pollinatimg insects, with special reference to results of experiments performed showing the possibility of red clover being pollinated by bees other than bumblebees and honey bees.

This investigation was done under the direction of Dr. J. W. Folsom and to him I desire to express my thanks for his constant advice and suggestions and for certain data on pollination. I desire also to express my thanks to Mr. W. P. Flint for putting at my disposal the records of observations that were made during the last two years, 1919 and 1920. My thanks are also due to Dr. C. P. Alexander for the identification of certain specimens.

II. METHODS

These observations, which were begun in the latter part of April, 1921, were made on a clover field on the South Farm of the University of Illinois. This field is one of a series of plots which is being used by the experiment Station in crop rotation investigations and bears the serial number 700. It is subdivided into eighteen one-fifth acre plots, each of 2 by 16 rods, numbered consecutively from 741 to 758 inclusive, from north to south. Its total area is three and three-fifths acres. It was sown to clover in 1920 and was therefore in the second year of its growth at the time my studies were made.

In studying the abundance of the seed-infesting insects, a strip about four feet wide throughout the west edge of this field was swept with an insect net, usually every other day, on Tuesdays, Thursdays, and Saturdays, in the forenoon from 10 to 12 o'clock as a rule. The insects collected, which were desired for the purposes of this investigation, were picked from the net by means of a pair of forceps and put, in most cases, together in vials containing about 70 per cent. of denatured alcohol. They were then sorted out in the laboratory and the number of individuals of each species was counted and recorded.

In sweeping the field with the net, the pacing was made as uniform as possible, as the length of the steps has a direct relation to the number of strokes with the net, and a wide variation in the number of strokes in the sweepings would materially affect the results.

The data obtained on the variation in abundance in this locali-

ty of the insects studied are here presented in the form of graphs. In discussing the results, it is desirable to deal with each species separately.

III. DATES OF CLIPPING OF THE PLOTS

On May 10 the plants were forming buds. A week later, May 17, the heads were beginning to show the pink, and some were in full bloom on May 19.

May 19, plot 741 was clipped and was again clipped on June 13.

Plot 742 was clipped on May 31.

June 16, plots 743 and 745 to 758 inclusive, were clipped, including the strip along the west edge of the field that was being swept.

Plot 744 was left unclipped.

Herafter only plots 741 to 749 inclusive, will be considered, as these were the only plots from which seed were extracted, the plants on plots 750 to 758, inclusive, being cut for hay.

Thus of all the plots, only one, No. 741, was double-clipped; one plot, 744, being left unclipped, and all the others single-clipped.

IV. RELATION OF CLIPPING TO NUMBERS OF INSECTS

The clipping on June 16 of the strip that was being swept necessarily affected the collection of insects, especially the clover seed-chalcid. It did not, however, affect the seed-midge, as the flies had already disappeared two weeks before June 16. It affected the lesser clover leaf-weevil to some extent. More will be said on this subject beyond.

Because of the clippings, the sweeping of the strip was discontinued, as the number of individuals of each species collected therefrom would be too low, and the figures obtained would not give a good indication of the abundance of each species. Instead of sweeping this area, a strip about 24 feet long and of the same width as the original strip, four feet, on the north edge of the unclipped plot, plot 744, was swept and the figures obtained were used in computing the number of individuals for the original strip. An objection to this method is that the figures would be too high, especially in the case of the chalcids, as their tendency would be to concentrate upon the plots which were not clipped on June 16, where they could find heads in which to oviposit.

On July 7, the clover heads in plot 742 were in just the right condition for oviposition by the chalcids. The insects were then observed in numbers as in plot 744. Beginning July 7, a strip in plot 742 of the same size as that in 744 was swept, the sweeping in 744 being continued until July 21. The clover on 744 was cut for seed on July 30.

The averages of the collections from the two strips from July 7 to 21 were taken and the figures were used in computing the number of individuals for the original strip. The computations of the number of individuals for the original strip from July 21 to August 3 were based on collections from the strip in 742 alone.

Beginning August 3, a strip about four feet wide along the west edges of plots 745 to 749 inclusive was swept. The sweepings in plot 742 were continued, however, until August 18.

August 20, the clover on plots 741, 742, 743, and 745 to 749 inclusive was cut for seed. Beginning August 20 and thereafter the

entire original strip was swept.

V. DISCUSSION OF THE ABUNDANCE OF PESTS STUDIED

1. THE CLOVER SEED-MIDGE

(Dasyneura leguminicola Lint.)

Graph No. I shows the results obtained for the clover seed-midge. In central Illinois two full broods of the seed-midge are known. There is also a feeble third generation of flies which properly belongs to the May, or first, brood of the next year. The midges of the first generation during the past season were first collected on May 10, two individuals having been collected on that day with the net. On May 12, ten were collected; then there was a drop in the collections to three and two on May 14 and 17 respectively. The number collected jumped suddenly to forty-five on May 19 and then, on May 21, to one hundred and twenty-six, the maximum number collected. The drop in the number collected three days later, May 24, was as sudden as the rise, fifty-two having been collected on that day. On May 27 and 28, the midges were scarce; only one individual being collected on each of these days. The flies disappeared three days later, as the record shows.

The flies of the second generation began to appear about August 18, but were not abundant, five being the maximum number collected, and this occurred on August 25. This scarcity of flies was probably due to the clippings of the clover.

Thus, according to these observations, the midges were on the wing during the past season on May 10, reaching their maximum abundance on May 21, and disappearing after a week. The flies of the

first generation were thus on the wing for a period of approximately three weeks. The dates of appearance and of maximum abundance of the midges for the past season are rather early, considering previous observations for this locality. Dr. J. W. Folsom, in 1907, did not find the midges until May 15; they were common May 23, but did not reach their maximum abundance until May 30. Dr. Folsom attributed this to the lateness of the season that year, due to prolonged cold weather in April. In 1903 to 1906 inclusive, according to Dr. Folsom, the dates of maximum abundance were May 24 and 25, the time when the young green clover heads were most abundant. Unpublished observations by Mr. Spooner, under the direction of Mr. W. P. Flint, entomologist of the Natural History Survey, for the last two years, 1919 and 1920, show that in 1919, the flies were on the wing May 15, reaching their maximum abundance May 31 and disappearing June 3. In 1920, the date of appearance was late, the midges having been collected first on May 24. They reached their maximum abundance two days later, May 26, and disappeared after two weeks. Judging from these observations, the adults of the first generation may be expected in the second week of May or earlier.

The larvae of the first generation of the clover seed-midge were at work in the heads during the last days of May and during June. On June 4, the midges were abundant in the heads, 26 larvae having emerged from ten clover heads in full bloom. It is known that the larvae of the first generation may be found working in clover heads as late as the first week of July and even later.

A fairly accurate estimate of the amount of infestation by the midge larvae may be obtained by putting the infested clover heads

in a corked bottle and then counting the number of larvae that emerge from the clover heads. Mere examination of the heads by tearing off the florets will not show all of the larvae or may show none at all. In the case referred to previously, in which 26 larvae were obtained from ten heads, mere examination of the heads by tearing off the florets disclosed only three larvae, whereas when these same heads were put in a tightly corked bottle a total of 26 larvae was obtained.

The larvae of the flies of the second generation, according to Dr. Folsom, are most abundant during the last two weeks of August and the first two weeks of September, and it is during this time that they do most damage. Most of these larvae pass the winter either as larvae or pupae. A few of the larvae, however, are said to attain their full growth early enough to produce flies in early September or even later.

As to when the larvae of the second generation leave the heads to pupate in the soil, I do not find any definite statement for central Illinois. They may remain in the heads until the middle of October or even later, depending upon the weather. In 1919, the writer observed the larvae still common in clover heads on October 3, and in 1921 they were still found in considerable numbers on October 6.

In the laboratory, with artificial heat, the flies may emerge any time during the fall and winter. Thus, in 1919, larvae collected on October 3 gave adults on October 27 and November 3; and in 1921, larvae collected October 6 gave adults in December and in January 3, 1922.

2. THE CLOVER SEED-CHALCID

(*Bruchophagus funebris* How.)

Adults of this species were first collected on May 19, six individuals having been caught with the net on that day. There was a gradual increase for two succesive sweepings on May 21 and 24, the number of individuals collected being 14 and 16 respectively. Then a drop in the collections followed, the curve maintaining an almost constant level for three successive sweepings, and then dropping suddenly, and eventually to zero on June 13.

As has been stated elsewhere, the strip which was being swept was clipped on June 16. This affected the collections, as the chalcids naturally concentrated on plots 741, 742 and 744, especially on 744, where there were heads suitable for oviposition. The concentration of the chalcids on plot 744 may be taken as a reason for such a sudden rise in the collection, as shown in curve A, graph II, from zero on June 13 to over 200 on June 22. The figures obtained from the sweepings in plots 744, 742 and the strip comprising the west edges of plots 745 to 749, inclusive, were used in computing the number of individuals that would have been collected from the original strip. It is from such computations that curve A was plotted.

The portion of curve A beginning June 16 and continuing up to August 18 should be taken as theoretical, being derived from the collections from the strips in plots 742, 744, and 745 to 749, inclusive.

Curve B was plotted from actual collections from plots 742 and and 744, while curve C from the actual collections from the strip comprising the west edges of plots 745 to 749, inclusive.

It should be added that the remaining part of curve A, beginning August 20, represents actual collections from the whole original strip.

As the preceding observations show, the chalcids were on the wing May 19. According to Dr. Folsom (1909), the chalcids as a rule do not begin to emerge until May 15. Some observations in previous years, however, showed the date of emergence to be later than my record. Thus, in 1907, Dr. Folsom found no adults until May 23, and in 1920, Mr. Spooner did not collect the adults until May 26. On the contrary, in 1919, Mr. Spooner collected the adults more than two weeks earlier, May 10, which is rather early.

As the curves show, the chalcids were most abundant after the middle of June, the maximum number collected occurring on June 23, after which there was a sudden decrease in the number. The insects were fairly common throughout July up to the middle of August, after which the adults became scarce. It is known that the chalcids can be found from May 15 to October 15, or later, especially on volunteer clover, where they can find heads at any time in just the right condition for oviposition. During the past season no individuals were found after October 15, except one, and this was collected on October 27.

3. THE CLOVER SEED-CATERPILLAR

(Enarmonia interstinctana Clem.)

The moths of this species were not present in such numbers as to constitute an important pest in 1921. Graph III shows that the largest number of moths collected in May was three. On June 22 and 23, the number collected on each day was eight. These numbers for June, however, were not actual but computed, being based on the numbers collected from the fifty sweeps made in plot 744. After June 23, no more moths were collected except on August 12 and 16, three moths having been collected on each of these days. These figures for August, like those for June, were computed, being based on the collections from the strip comprising the west edges of plots 745 to 749 inclusive.

In 1920, the moths were present in large numbers. They were most abundant during the last two weeks of May and the first week of June, the maximum number collected being more than five hundred. They were present in much fewer numbers in July, and fewer still in August of that year.

On June 30, 1921, a hymenopterous parasite emrged from a larva collected on June 22, 1921. This parasite was identified by Mr. H. L. Vierick as belonging to the genus Microbracon.

VI. RELATION OF CLIPPING AND ABUNDANCE OF PESTS TO THE YIELD OF SEED

Plot 741 was clipped on May 18, at a time when the plants were forming buds, when the clover seed-midge adults were on the wing, and when the chalcids and lesser clover leaf-weevil adults and larvae were not yet abundant. It was again clipped on June 13, at a time when the clover seed midge larvae were at work in the clover heads and when the clover leaf-larvae and adults were abundant. At this time the chalcids were not as yet abundant. Plot 741 was thus double-clipped.

Plot 742 was clipped on May 31, at a time when the clover seed-midges had already disappeared but when the larvae of this species and of the lesser clover leaf-weevil were at work in the clover heads. At this time also, the chalcids were not yet abundant.

Plots 743 and 745 to 749 inclusive were clipped on June 16. This time the clover seed-midge larvae were at work in the clover heads.

Plot 744, as has been stated previously, was left unclipped.

The following table shows the yield in seed of these different plots in bushels per acre.

Plot No.	Yield in bushels per acre.
741	0.46
742	0.64
743	0.80
744	0.51
745	0.72
746	1.00
747	1.04
748	0.92
749	0.95

Examination of the preceding table shows that the acre yields

by plots are extremely low, far below the average in Illinois.

One reason for these extremely low yields is no doubt the scarcity of bumblebees during the past season. A second reason is probably the weediness of the field as a whole, particularly plots 741, 742 and 744, and a third reason is the somewhat thin stand of clover that was present.

Comparison of the preceding yields shows several things. The double-clipped plot, 741, and the unclipped plot, 744, gave the lowest yields, being 0.46 and 0.51 bushels, respectively. The plots which were single-clipped, 742, 743, and 745 to 749 inclusive, gave each a higher yield per acre, the yield ranging from 0.64 to 1.04 bushel per acre, the average being 0.88 bushel.

The reason for the lower yield in the unclipped plot, 744, is probably the fact that the seed-infesting insects, the chalcids especially, concentrated their attacks on it after most of the other plots were clipped.

VII. THE LESSER CLOVER LEAF-WEEVIL

(Phytonomus nigrirostris Fab.)

1. General Importance

It seems desirable, in view of the importance of the insect, to give a brief historical account of its origin and also some data as regards its distribution in North America, particularly in the United States, so far as could be gathered from the literature.

2. Origin and Distribution

The lesser clover leaf-weevil invaded North America from Europe, where it is of general occurrence. The date of its introduction into America is not definitely known. F. M. Webster, who published a detailed historical account of the insect in 1909, makes the assumption that it first gained a foothold in New Brunswick and elsewhere in extreme eastern Canada, his basis for this assumption being that it first became destructive in those sections of North America. From these regions the pest spread to other parts of the continent by natural agencies. Webster also states that Hubbard and Schwarz collected the insect in Massachusetts during the winter of 1873-74, and that Blanchard, one of the oldest and most careful collectors of New England Coleoptera, is of the opinion that it may have occurred in Massachusetts as early as 1865. Provancher, in 1877, described it from Quebec under the name of Erirhinus viridis. How long the species had been known to Provancher prior to his description of the insect is not known. Since 1873-74, the insect has gradually extended its territory. Titus (1911) showed that the range of the species in that year comprised southeastern Canada (New Brunswick,

Ontario and Quebec, including Prince Edward Is.), the New England States, and extended westward into Indiana and as far south as the southeastern coast of Virginia. Webster (1909) discussed the possibility of the insect having spread southward through the agency of the Gulf Stream, floating on debris carried from the beach. Titus, however, thought this to be improbable, stating that many persons have tried the effect of salt water on insects and found that it is rare for the latter to survive longer than a few hours. That the insect reached the southern coast by gradual flight, passing a little farther southward year by year, or that it was carried by trains, seemed to Titus to be more probable. In support of the first possibility, he mentioned Houghton's report on the spring flight of the species in Delaware.

3. Present Distribution of the Species in in the United States

The latest paper giving a detailed account of the distribution of the species was that by Titus in 1911. According to him, the distribution of the insect in the United States, as then recorded, embraced the following states: Maine, New Hampshire, Vermont, Massachusetts, Rhode Island, Delaware, Connecticut, New York, New Jersey, Pennsylvania, Maryland, District of Columbia, Virginia, Michigan, Indiana, and Minnesota. Since 1911, so far as I have ascertained, there has not been any published record of the insect in other states except one by Cooley, who recorded it from Montana in 1916. It is of interest to trace the migration of the insect since 1911. If it has been present in Montana since 1916, it is probable that the beetle has been present also in other states east of Montana.

4. Food Plants

In Europe, Algeria, Egypt, and in Asia Minor, it has been recorded as feeding on different species of Ononis, especially Ononis spinosa. Its other food plants in Europe are Buphthalmum salicifolium and Trifolium pratense. Trifolium agrarium has also been given as a probable food plant of the insect on that continent.

In North America, it is known to feed on red clover (Trifolium pratense), mammoth clover (Trifolium medium), alfalfa (Medicago sativa), crimson clover (Trifolium incarnatum), white clover (Trifolium repens), and alsike clover (Trifolium hybridum).

5. The Species in Illinois

Mr. W. P. Flint, entomologist of the Illinois Natural History Survey, observed large numbers of the beetles in Urbana, Illinois, in the fall of 1919. The pest may have been present in Illinois some years before this, having escaped observation; for Titus, in 1911, recorded the insect as occurring as far west as the western boundary of Indiana.

Heretofore there has not been any published record and account of the insect from Illinois since Mr. Flint found it in 1919. During 1920, a study of its abundance was made by Mr. Spooner, under Mr. Flint's directions. The following observations were made by the writer at Urbana, Illinois, on a clover field on the University Farm.

6. Nature and Extent of Injury

Early in May, 1921, the writer found the young larvae of this

species in the leaf axils of the clover plants, feeding on the young leaf buds and riddling them with numerous holes, which increased in size as the leaves grew. Usually only one larva was found in one leaf bud, but rarely two. In no instance has the writer found three.

In flower heads the larvae feed near the bases of the florets. Only one larva is usually present in one head, though occasionally two may be found. A larva eats only a few of the florets and prevents these from forming seeds. The greatest damage done by the larvae, however, is in the newly developing heads, as the larvae feed on these as they appear and the damage results in the malformation of the flowers. Just how much damage is done by the insect in this manner, the writer has not been able to determine, but Mr. Flint states that fifty per cent. of the heads may be made abnormal by the larvae in this way when the latter are present in large numbers.

The beetles feed on the leaves and make numerous holes, particularly near the midribs, seeming to show preference for the young growing leaves. The adults have been observed by the writer resting on clover heads in full bloom, but he has not happened to observe them feeding on the florets.

On May 17, 1921, heads were picked at random in order to determine the amount of infestation by the larvae. Out of thirty-seven heads, thirteen were found to contain larvae, i.e., thirty-five per cent. of the heads were infested. Of sixty-four heads picked at random from volunteer plants growing near First Street, Champaign, twenty-six, or forty per cent., of the heads were found to be infested. Out of these twenty-six heads infested, two heads contained two larvae each, one three larvae, and all the others one larva each.

7. Life History and Habits

In Urbana, on April 12, 1921, active adult beetles were found together with adults of the clover root-curculio (Sitones hispidulus).

On April 28, sweepings were begun in order to determine the abundance of this insect at different periods of the season, as well as that of the clover seed-chalcid, clover seed-midge, and the clover seed-caterpillar.

On May 7, young larvae of this species were noticed by the writer for the first time, feeding on the young leaf buds of red clover. The presence of the larvae was easily detected by means of the feeding holes and the excreta on the buds. Several larvae were collected and taken to the laboratory, where they were confined in vials and supplied with fresh clover leaves every day, the vials being plugged with cotton to allow circulation of air. The following table shows partly the life history of the insect, as studied by me:

Larvae	Date of spinning cocoon	Date of pupation	Date of emergence
1	May 16	May 18	May 23
2	May 16	May 17	May 23
3	May 16	May 17	May 23
4	May 14	May 17	May 23
5	May 16	May 17	May 23

The preceding data show that the pupal stage is five or six days, and that the larva pupates one to three days after spinning its cocoon. The time spent by the insect in the cocoon, according to the preceding records, was, on the average, eight days. Herrick and Detwiller (1919) found that, in Ithaca, New York, the period spent by the insect in the cocoon, from the time of spinning to the

emergence of the adult, was on the average, fourteen days, which is almost twice as long as the record in Urbana. Herrick and Detwiller made their observations during the last days of June and during July, 1919.

Of the larvae collected on May 7, two were confined together in one vial. Both of these spun cocoons on May 12. One pupated on May 13 and the other on the following day. Both beetles emerged on the same day, May 20, with a difference of a few hours, the one pupating first, of course, emerging first. The first adult, after having emerged, ate all of its cocoon and after having done this it ate also the caudal end of the cocoon of the other insect, which was already a fully developed adult but still in the cocoon.

On May 14, six adults were collected and placed in a vial. These were transferred to potted clover plants in the laboratory and a chimney cage put over them. Two of these beetles were in copula upon transference. On May 16, in addition to numerous feeding holes, punctures were observed in the leaves, and an examination of these punctures showed the presence of minute elliptical eggs, which were those of the beetles. The punctures in which the eggs were laid were made mostly through the upper epidermis of the leaves, and this is probably the normal mode of oviposition in the leaves. The eggs were mostly under the lower epidermis, and could be detected and located easily even with the unaided eye, since they formed swellings on the lower surface of the leaves. As a rule, only one egg is laid in each puncture. In some instances, however, two eggs were observed together in the same puncture. A case was observed in which two eggs attached end to end were simply laid on the surface of the leaf. A case was also noticed in which an egg was only partly inserted in the

puncture.

Oviposition probably takes place at night, the insect remaining rather inactive during the day.

The following record was made of the life history of the insect in the laboratory:

May 14----------6 adults in captivity.
May 16----------Eggs laid.
May 17----------Eggs have turned dark.
May 23----------Eggs hatched.
June 4----------Larvae formed cocoons.
June 6----------Larvae pupated.
June 11----------Adults fully developed but still in the cocoons.
June 12-13----------Beetles emerged.

As shown by the above data, the eggs turn dark within one day after oviposition and hatch in seven days. The larval and the pupal stages are fourteen and five days respectively. Thus the insect spends a large part of its life in the larval stage. The life cycle, from the date of oviposition to that of the emergence of the adult, is twenty-seven days. The life-cycle of the insect out-of-doors probably differs from that in the laboratory, on account of differences in temperature, moisture, and other factors.

As mentioned previously, the eggs of the insect in the laboratory were laid in punctures in the leaf. In the field, eggs are certainly not normally deposited in the leaves. Wildermuth (Webster, 1909) examined hundreds of leaves without finding a single egg on any, but he had no difficulty whatever in finding the eggs under the epidermis of the basal leaf sheaths, more often in groups of three.

Webster (1909) made the statement that cocoons were usually spun among the full-blooming heads, although one cocoon was found on a clover leaf by Caudell. Contrary to this observation, the present writer has found the cocoons to be more abundant attached to the

lower surface of the basal foliage and especially among the dried leaves on the surface of the ground, the cocoons being also attached to these dried leaves.

The beetle is brown when fully developed, the color being distincly visible through the coarsely woven silky cocoon. Records in the laboratory show that a day usually elapses after the adult is fully developed, before the beetle eats its way out of the cocoon. It assumes its normal green color at least three or four days after emergence. The temporary brown color has a significance in connection with the determination of the abundance of the adults in the field at different periods of the growing season and of the number of generations, as will be seen subsequently in this paper.

8. Abundance

Graph No. IV shows the variation in abundance of the adults as determined by sweepings. The adults were still scarce between April 28 and May 24, the maximum number collected during this period being thirty-nine, on May 12. The adults began to be collected in numbers on May 27, the number collected on that day being ninety-one. The number collected dropped to forty on May 28, increasing to one hundred and forty-one on May 31, and then to one hundred and seventy-four on June 2. There was a drop again to seventy-six on June 4, but this was followed by a sudden rise to four hundred and thirty-seven, the maximum number collected, on June 9. The decrease in the collections on June 13 was also sudden, the number collected on that day being one hundred and ninety. On June 16, as has already been stated, the strip which was being swept was clipped. The portion of the curve from June 22 up to August 20 does not represent actual

collections, being computed chiefly from the figures obtained from the strip in plot 744, the unclipped plot.

Thus, according to the preceding observations, the period of abundance of the beetles extends from the last days of May to about the last days of June, i.e., approximately one month. The beetles were scarce during July and August and thereafter.

On May 27 newly emerged adults were collected for the first time during the season. Then there was an increase in the collections from that date onward. These newly emerged adults were of the new brood, while the beetles collected before May 27 were probably of the last year's brood. Out of ninety-two beetles caught with the net on May 27, thirty-three had recently emerged, as indicated by the brown color. Recently emerged adults continued to appear until June 30, after which no more were caught with the net. Out of the maximum number of adults collected on June 9, 384 had evidently recently emerged, and probably almost all of those collected on that day were of the new brood.

The following table shows the number of newly emerged adults collected during the period of greatest abundance:

Date	No. collected	Newly emerged
May 27	92	29
" 28	40	27
" 31	141	133
June 2	174	144
" 4	76	64
" 9	437	384
" 13	190	123

The beetle is, as a rule, nocturnal in its habits, resting on the ground under rubbish and in between the bases of the stems of the plants during the day, and mating and doing most of its feeding at night. Some adults, however, remain on the plants during the day,

and a large number do so when the ground is very moist. This, in a way, affects the number of specimens collected with the net. On a day following a rain an unusually large number of adults can be collected with a net.

After the clipping of most of the plots on June 16, the beetles were abundant in the stubble and under the cut hay, together with Sitones flavescens and Hypera punctata. On June 22, the new leaves were observed to be greatly damaged by these these three species, being riddled with numerous holes. There is no doubt that the combined injury by these three species may do much to reduce the hay crop and also to retard the growth of the clover plants.

It seems reasonable to infer from the first appearance of the larvae in the field, the records of the abundance of the larvae, and from life history records in the laboratory, that the beetles begin to lay their eggs in the latter part of April and continue to oviposit through May. The larvae were most abundant and did most damage after the middle of May up to the middle of June, and were scarce toward the last days of June. The period of emergence of adults in the field, as far as my observations showed, ranged from May 27 to June 30.

On June 27, ten adults were confined in the laboratory on potted plants, but these did not lay eggs. Throughout July and August, no larvae were observed in the field, showing that the insect had ceased to breed during these months, although records by Mr. Spooner show that the larvae have been collected as late as July 17 and 21. There is probably only one generation during May and June, but oviposition is prolonged over a considerable period. From May 7 up to June 15, the larvae were found in all stages of development.

On September 20, a newly emerged adult and a cocoon were collected by the writer. Again, on October 6, a newly emerged beetle was found. All of these records are evidences of second generation of beetles, although the insects of the second brood are not abundant.

The insect hibernates as an adult on the ground under the dead plants and other rubbish in the field and in between the stems of the living plants.

9. Natural Enemies

Two insects are known to be parasitic on the larvae of Phytonomus nigrirostris, namely, a small fly, Anisia species, near variabilis Coq., and a hymenopteron, Bracon sp., both of which were reported by Webster in 1909.

The larva of this insect is attacked by a fungus, Entomophthora sphaerosperma Fres., which is also the worst enemy of the larva of Hypera punctata. As to the manner of the attack of this fungus, on the larva of Hypera punctata, Titus says: "The fungus develops in the body of the host, as a network of branching mycelia, some of the branches push through the ventral wall and become attached as rhizoids to some surface; over the body is formed a gray velvety coating of fine threads which have penetrated the skin; on the tips of some of these are formed conidia; from these come temporary spores which are shot away from some distance and may thus alight upon another host and begin to grow. Resting spores develop inside the host and probably may thus live over until the next season.

"The sick larvae crawl up the plants during the night, ascending as high as possible; if on a slender stem or a grass blade they coil

themselves about it in a horizontal position.

"Arthur (1885) states that they die by noon, remaining in this position, and during the late afternoon have changed to a velvety gray. By the next morning the larva is only a blackened shriveled mass."

According to Dr. Folsom, who published a detailed account in 1909 of the attack of the fungus on the larva of Hypera punctata as observed by him in Illinois, the fungus needs damp and not too cold weather for its development, and affects the larvae in April and May and again in October and November, but not during the winter. Under these favorable conditions, the disease may kill the larvae of Hypera punctata by the wholesale.

The manner of the attack of the fungus on the larva of Phytonomus nigrirostris is probably similar to that on the larva of Hypera, and the extent of its infestation, under favorable conditions, may be equally great.

On June 2, 1921, four cocoons of Phytonomus nigrirostris were collected in the field by the writer and placed in a vial plugged with cotton. On June 16, 1921, a hymenopterous parasite had emerged from one of these cocoons. This was identified by Dr. C. P. Alexander, entomologist of the Illinois Natural History Survey, as Spilochalcis torvina Cress. and this determination was later confirmed by Mr. Henry L. Vierick.

So far as I have ascertained from available literature, this is the first record of the species as being parasitic on Phytonomus nigrirostris. Titus (1905) reported having reared the chalcid from the sugar beet crown borer, Hulstea undulatella Clemens, and also from the pupa of an unidentified tortricid from Chino, California.

Cresson described the chalcid and gave Massachusetts, Connecticut, Illinois, and Texas as its habitat. Thus, according to the preceding records, the parasite is widely distributed in the United States. Cresson's technical description of the species follows:

> Female--Black, opaque; spot between antennae, transverse beneath each posterior ocellus, spot on anterior orbits; narrow posterior orbits, and the large mandibles except tips, pale yellow; thorax densely rugulose; prothorax with four whitish dots on anterior margin—two on each side and two approximately above —and a reddish spot near each posterior angle; mesothorax with a short line on each side of middle lobe and two spots on posterior part of each lateral lobe, pale orange yellow; scutellum with a rounded pale yellowish spot on each side, tegulae pale yellow, fuscous posteriorly; wings hyaline; legs black, sericeous; tips of anterior femora, their tibia at tips and within and all the tarsi, except tips, yellowish white; posterior coxae entirely black; their femora black, inner side with a yellowish apical spot, outer side with a spot at base beneath, a spot at tip above and a transverse tortuous band near apex, pale yellowish, sometimes irregularly margined with brown, and interrupted into spots, femoral teeth minute; posterior tibiae black, with a yellowish annulus at tip and another near base; abdomen subsiform, smooth and polished, first segment with a whitish spot on each side between middle and apex, sometimes wanting and the second segment with a large spot of same color on each side at base, sometimes the fifth segment has lateral pale spot; petiole robust, scarcely half the length of posterior coxae. Length .15-.20 inch. Hab.—Massachusetts, Connecticut, Illinois, Texas. The markings on posterior femora vary considerably.

This hymenopterous parasite was collected by the writer at various times in the field. The following data show the number collected:

Date	No. collected
June 22	2
June 23	2
August 4	1
" 12	1
" 24	1

It will be worth while to ascertain the percentage of parasitism in future studies in this locality.

On June 16, 1921, a dead larva was found, on examination of which a hymenopterous larva was found attached transversely to the

ventral aspect of the third segment, but unfortunately this parasite succumbed to dryness in the laboratory.

The carabid beetles of the species Calosoma calidum, were observed in the field. On July 7, one beetle was taken alive to the laboratory and placed in a glass jar, and with it were placed the following insects:

Four adults of Phytonomus nigrirostris

Two adults of Sitones hispidulus

Three caterpillars, species unknown.

On July 8, all of these insects were found to have been devoured by the beetle, leaving only such apparently unedible portions of the bodies of the adults of Phytonomus and Sitones, as the elytra and portions of the legs. It seems probable, then, that this carabid preys on Phytonomus nigrirostris in the field also.

VIII. POLLINATION OF RED CLOVER

1. INSECTS OBSERVED WORKING ON CLOVER HEADS

Red clover is a plant which is entirely dependent upon insects for it fertilization. Of the insects that pollinate red clover, the bumblebees are the most important. It is estimated that the United States is indebted to bumblebees to the amount of $60.000,000 annually for their work as pollinators of red clover, to say nothing of other farm crops.

The abundance of bumblebees in a particular locality varies from year to year. During the past season, for example, bumblebees were not observed in numbers in clover fields at Urbana, and this certainly accounts to a large extent for the extremely low yields of red clover here reported.

The importance of bumblebees as pollenizers depends upon their numbers when the clover field is in full bloom. In Illinois, bumblebees are considered most important pollenizers of the second crop of clover, for the workers are abundant in late July, August, and September. They are not important as pollenizers of the first crop, for in June the only bumblebees on the wing are a few queens that have survived the winter and these do not do much pollinating as they stay most of the time in the nest. It is a well known fact, therefore, that the first crop of clover is not pollinated by bumblebees to any appreciable extent.

In a recent paper, Dr. Folsom made the statement that twenty years ago in Illinois, it was taken for granted that there was not enough seed in the June crop to pay for its extraction. Since then, however, farmers in the state in increasing number have found it

profitable, now and then, to cut the June crop for seed instead of hay, and have obtained ~~reasonable~~ yields of from one half a bushel to two bushels of seed, and possibly more, per acre.

This being the case, the question as to how the June crop is pollenized needs to be answered. Dr. Folsom has learned from various sources that in a few instances the yield of seed had occurred in a region where Italian honey bees were common and had been observed working on the flowers. Dr. A. D. Hopkins and Dr. Folsom have both found out that honey bees of this race are no doubt important pollenizers. There ^were, however, cases reported in which yields of clover seed had been obtained in places where the honey bees did not occur, so that in the words of Dr. Folsom, "there still remained some mystery as to how the June crop is pollenized."

Now, according to Dr. Folsom, Mr. W. P. Flint has on several occasions observed a species of bee belonging to the genus Tetralonia sucking the florets of red clover in June, and has several times received the same bee from observant farmers, who noticed its abundance in the clover fields.

After the middle of May, 1921, the writer observed a species of bee working on the heads of clover. This bee was later identified by Vierick as Tetralonia dilecta Cress. Its presence in large numbers in the field attracted attention to such an extent that Dr. Folsom and ^the writer undertook to make some observations and perform experiments in order to find out whether this species of Tetralonia and other species observed in the field pollenize red clover or not. On May 24, Dr. Folsom took a root cage with parallel glass sides to the clover field; in it was placed a solid row of clover heads in full bloom and then several individuals of Tetralonia dilecta were introduced

into the cage. Some of the bees worked on the blossoms, their operations being studied under a hand lens. Examination under the microscope of the mouth parts of specimens collected from the field that had previously been observed working on the heads of clover showed the presence of many pollen grains, especially on the mentum, but also on the maxillae. As a matter of fact, pollen grains were observed sticking almost everywhere on the ventral aspect of the mouth parts, these pollen grains being indistinguishable from those of red clover.

Tetralonia dilecta Cress. belongs to the family Euceridae, a family of solitary digger bees with but one male and one female to each nest. The two sexes of the species are so different from each other in general appearance, the female resembling the honey bee, that it may be mistaken for the latter, so that one who is not familiar with the sexual individuals will take them to be distinct species. For instance, we erred at first in calling the male Melissodes obliqua Say. For these reasons, it seems desirable to give in this paper brief descriptions of the sexes in order to enable the collector or observer to recognize them in the field.

Male. The clypeus is yellow and is sparsely pubescent. The pubescence of the thorax and of the legs is usually of a much lighter color than that of the female, being light yellow or nearly grayish. The antennae are longer than those of the female, reaching to the base of the abdomen. The male measures about 1.2 cm. in length.

Female. The clypeus is black and more hairy than that of the male. The pubescence of the thorax and of the legs is brownish yellow. The white transverse bands of hairs on the abdomen are more

pronounced than those of the male. The antennae extend to about the middle of the thorax a little caudad of the base of the anterior wing. The female measures from 1.5 to 1.7 cm. in length.

Specimens of Tetralonia dilecta were first observed and collected by the writer on May 17, 1921, when the first heads of clover were just beginning to bloom. On May 24, the bees were abundant and a few days after this Dr. Folsom and I began our experiments. On June 30, the bees had disappeared, showing that the period of activity of the species was approximately forty-four days.

A second bee of importance, Melissodes bimaculata Le P., appeared on July 5. This bee has also been observed working on the florets of clover and examination of the mouth parts showed the presence of pollen grains of red clover. This is a black bee, belonging to the same family as Tetralonia dilecta Cress. As in Tetralonia, the male has longer antennae than the female, as is characteristic of the family to which these insects belong.

Male. The male is slightly smaller than the female, measuring about 1.1 cm. The clypeus is yellow. The pubescence of the middle and posterior tibiae and the metatarsus of each posterior leg is white. The male lacks the two spots on the abdomen, which are present in the female.

Female. The female measures from 1.2 to 1.3 cm. in length. The clypeus is black. The posterior legs are more distinctly pubescent than the corresponding legs of the male, and their pubescence is brownish yellow instead of white, as in the male. The fourth dorsal abdominal segment has an apical patch of white hairs on each side; hence the name of the species.

Individuals of this species were first observed in the field on

July 5, but were not abundant then. On July 9, the bees were present in large numbers and remained so until August 5, after which they became scarce, disappearing on August 13. The period of activity of this species, therefore, was approximately forty days.

Besides these two species that have been described, there are other bees of less importance that were observed working on the heads of clover. Among these are two species of Megachile, latimanus Say and brevis Say, both of which belong to the family Megachilidae, a family of leaf-cutters. The members of this family have the habit of making cells for their young out of neatly-cut pieces of leaves. Of the two species mentioned, brevis was observed to be present in larger numbers and seems to be the better pollinator of the two. Megachile latimanus is the more alert, and is most of the time on the wing, and is easily disturbed by the approach of the collector. This species has the habit of darting swiftly past the collector, and it was while the insects were in the act of darting that the writer caught all of his specimens.

Small bees of the genus Halictus were also observed by the writer working on the heads of red clover.

A species of fly , Exoprosopa prorsa, was likewise observed sucking the florets of clover. This fly was first collected by the writer during the past season on July 19. It was present in considerable numbers August 16, after which it became scarce, disappearing on August 25. Thus its period of activity was approximately forty days.

Examination of the mouth parts of this fly failed, however, to show the presence of pollen grains. This is due to the fact that the mouth parts are smooth and destitute of setae to which the pollen

grains may attach themselves. The absence of pollen grains, however on the mouth parts is not sufficient evidence that the fly does not pollinate clover to some extent.

In addition to the insects that have already been mentioned, thrips were found in great abundance in clover heads and these insects are thought to pollinate red clover accidentally.

2. COMPARISON OF THE ACTIVITY OF THE BEES

With the object of comparing the activity of the different bees observed in the field, data were obtained as regards the number of heads visited and of the number of florets sucked by them per minute. In order to have a sound basis of comparison, the observations were made at periods when most of the heads were in full bloom. The following tables show the data obtained.

Name of insect	No. of heads visited per minute	Total No. of florets sucked per minute	Average No. of florets sucked per head.
Bombus pennsylvanicus	6	39	6.5
	6	37	6.1
	6	26	4.3
	4	46	11.5
	4	32	8.0
	6		
	3		
	6		
	5		
	3		
	5		
	4		
	5	38	8.0
	5	28	6.0
	6	38	6.3
Average	5	35	7.0
Bombus separatus	8	42	5.2
	5	31	6.2
	8	38	4.7
	9	22	2.4
Average	7	33	4.5

Name of insect	No. of heads visited per minute.	Total No. of florets sucked per minute	Average No. of florets sucked per head
Tetralonia dilecta (Female)	8	10	1.2
	9	13	1.4
	9	12	1.3
Average	8	12	1.3
Tetralonia dilecta (Male)	6		
	5		
	4		
	3		
	1		
	1		
		36	
		18	
		12	
		10	
		12	
	8	13	1.6
	5	19	3.8
Average	4	17	2.7
Melissodes bimaculata	4	16	4.0
	3	13	4.3
	3	11	3.7
	5	16	3.2
	4	15	3.7
	2	15	7.5
	3	15	5.0
	7	19	2.7
	8	19	2.3
Average	4	15	4.0
Megachile brevis	2	12	6.0
	5	9	1.8
	3	9	3.0
	4	11	2.7
	3	9	3.0
	2	9	4.5
Average	3	10	3.0
Apis mellifera	5	17	3.4
	3	12	4.0
	8	17	2.1
	3	12	4.0
	6	16	2.6
	3	12	4.0
	6	14	2.3
Average	5	14	3.0

The preceding data may be summarized in the following table:

Name of insect	Average No. of heads visited per minute	Average No. of florets sucked per minute	Average No. of florets sucked per head
Bombus pennsylvanicus	5	35	7
Bombus separatus	7	33	4
Tetralonia dilecta (Female)	8	12	1
Tetralonia dilecta (Male)	4	17	3
Melissodes bimaculata	4	15	4
Megachile brevis	3	10	3
Apis mellifera	5	14	3

(Note: In figuring out the averages, decimals exceeding 0.5 were taken as unity.)

Although the preceding observations are somewhat meager, yet they are presented, for they give some idea as to the relative activity of the bees in pollination. The last table summarizing the data obtained speaks for itself. It appears that the bumblebees, Bombus pennsylvanicus and Bombus separatus, sucked more florets than the other bees here listed. B. pennsylvanicus visited from three to six heads per minute, the average being five, and sucked florets in each head ranging from twenty-six to forty-six, the average being thirty-five. The average number of florets sucked by this species per clover head is seven. B. separatus visited more heads per minute than B. pennsylvanicus, but sucked fewer florets per minute, per head. Of the sexes of Tetralonia dilecta, the female, as the figures show, visited more heads per minute than the male but sucked fewer florets in the same period of time. The male, therefore, of this species may be regarded as the better pollinator. There is not much appreciable difference, however, between the male Tetralonia dilecta and Melissodes bimaculata and the Italian honey bee, Apis mellifera, in this respect. Megachile brevis stands some-

what below these three last bees mentioned in the number of heads visited and in the number of florets sucked per minute.

3. EXPERIMENTS ON POLLINATION OF RED CLOVER

The statement has already been made that Tetralonia and Melissodes were observed thrusting the proboscis into the florets of red clover blossoms and that examination of their mouth parts showed the presence of numerous pollen grains, especially on the tongue and maxillae, which were indistinguishable from those of red clover. These observations, however, are not alone sufficient to justify the conclusion that these bees pollinate red clover. It was necessary to perform experiments to determine whether clover heads on which the bees had worked, and from which other insects had been excluded, would produce seeds. Clover plants with unopened buds were carefully dug up from the field and transplanted to flower pots of about twenty centimeters in diameter. All heads which were in full bloom were of course removed from the plants. These potted plants were placed out-of-doors on bigger pots of about thirty-four centimeters in diameter filled almost to the top with soil. The plants were then protected with cylindrical wire cages of about forty-eight centimeters in height and forty-five centimeters in diameter and with a mesh of one millimeter. When some of the heads began to bloom, bees were collected from the field and turned loose in the cage.

Experiment with Tetralonia dilecta Cress.

Clover plants with unopened buds were potted on May 25. On May 26, some of the clover heads were in bloom and Dr. Folsom put thirty individuals of T. dilecta into the cage. Some of the bees were ob-

served working on the flowers on the same day. After twenty-four hours, all of the bees had died. Five days later, May 31, the writer put four males and three females into the cage, and some of these were also observed working on the flowers. These bees also died after twenty-four hours. It was observed that the bees, soon after being turned loose in the cage, rested and crawled on the wire, especially near the top of the cage, and attempted to find their way out. In their flight within the cage, however, some of the bees struck the clover heads and in this way came to find them and work on the florets. For this reason, in order that the bees could find the flowers more readily, it was found necessary to raise the pots in such a way that the clover heads would be as close to the top as possible.

The writer did not have any difficulty in catching the bees in the field. In the act of sucking the florets, they did not seem to mind the slightest interruptions and were easily caught by the use of a wide-mouthed bottle.

On July 1, the clover heads had dried and were picked for seeds. From 789 florets, not counting undeveloped florets which could not have been pollinated, Dr. Folsom and the writer obtained 171 seeds, showing that twenty-one per cent. of the florets had been pollenized by Tetralonia.

Experiment with Melissodes bimaculata Le P.

Clover plants for this experiment were potted on July 12. Two days later, when some of the flowers were beginning to bloom, Dr. Folsom put seven individuals in the cage at 2:30 p.m., and he observed one of these working on the heads ten minutes later. On July

16, the writer introduced eleven bees, and on July 19, five more. Some of these were also observed working on the heads. In all a total of twenty-three bees had been introduced into the cage.

These bees behaved in the cage in much the same way as did the Tetralonia individuals, and like the latter did not live long in the cage. Most of them died after twenty-four hours.

Melissodes bimaculata is much more active on the wing than Tetralonia dilecta, and the author had some difficulty in collecting specimens for the pollination experiment by means of a wide-mouthed bottle and found it necessary to use the insect net.

On August 4, 1921, the writer picked the dried heads and examined them for seeds. As several bees had been observed working on the heads, Dr. Folsom and the writer expected to get some seed, but when the heads were examined the 216 florets counted gave only two seeds. Thus the results of this experiment were negative.

A second experiment, however, with the same insect gave much better results. Plants for this experiment were potted on July 21 and were given the same care as those in previous experiments. On July 23, some of the flowers were in full bloom and the writer put fifteen individuals of M. bimaculata into the cage. On July 25, sixteen more bees were introduced; thus thirty-one bees were used in this experiment. Many of these bees were observed working on the heads. On August 12, the heads were picked for seed. Seven heads gave a total number of two hundred and ninety-three florets, from which ninety seeds were obtained. This experiment showed that Melissodes bimaculata Le P. pollinates red clover.

Experiment with Exoprosopa prorsa

Plants for this experiment were potted on July 26. Two days later, July 28, when some of the heads were in bloom, the writer introduced ten flies into the cage. The flies, after a few minutes in confinement, stayed almost invariably on the sides of the cage. None of these flies was observed working on the heads. In the morning of July 29, only two flies remained alive; these were still alive on July 30, showing that these insects live a little longer in confinement than individuals of Tetralonia dilecta and Melissodes bimaculata. On July 30, twelve more flies were introduced into the cage. Some of these were observed working on the clover heads.

On September 16, the heads were examined for seeds. It is only necessary to mention that the nine heads obtained in this experiment produced no seeds.

Thus the results of this experiment were negative.

Experiment with Megachile brevis Say

As in the preceding experiment, the plants used were potted on July 26. Only two bees were used in this experiment but the insects worked very industriously on the clover heads. Like the other bees experimented with, individuals of M. brevis do not live long in confinement.

The results of this experiment were not conclusive, as only three seeds were obtained from twelve heads. It must be remembered, however, that the first experiment with Melissodes bimaculata gave similar results.

Controls

In connection with the preceding experiments, red clover plants with heads in bloom removed and having only unopened buds, were planted in two pots. These potted plants were used as controls. They were placed out-of-doors and covered with wire cages just as in the preceding experiments, but no bees were introduced into the cages. When the heads were picked and examined, one of the pots gave 357 florets, all of which were without seeds. The other pot gave 287 florets, which were likewise seedless.

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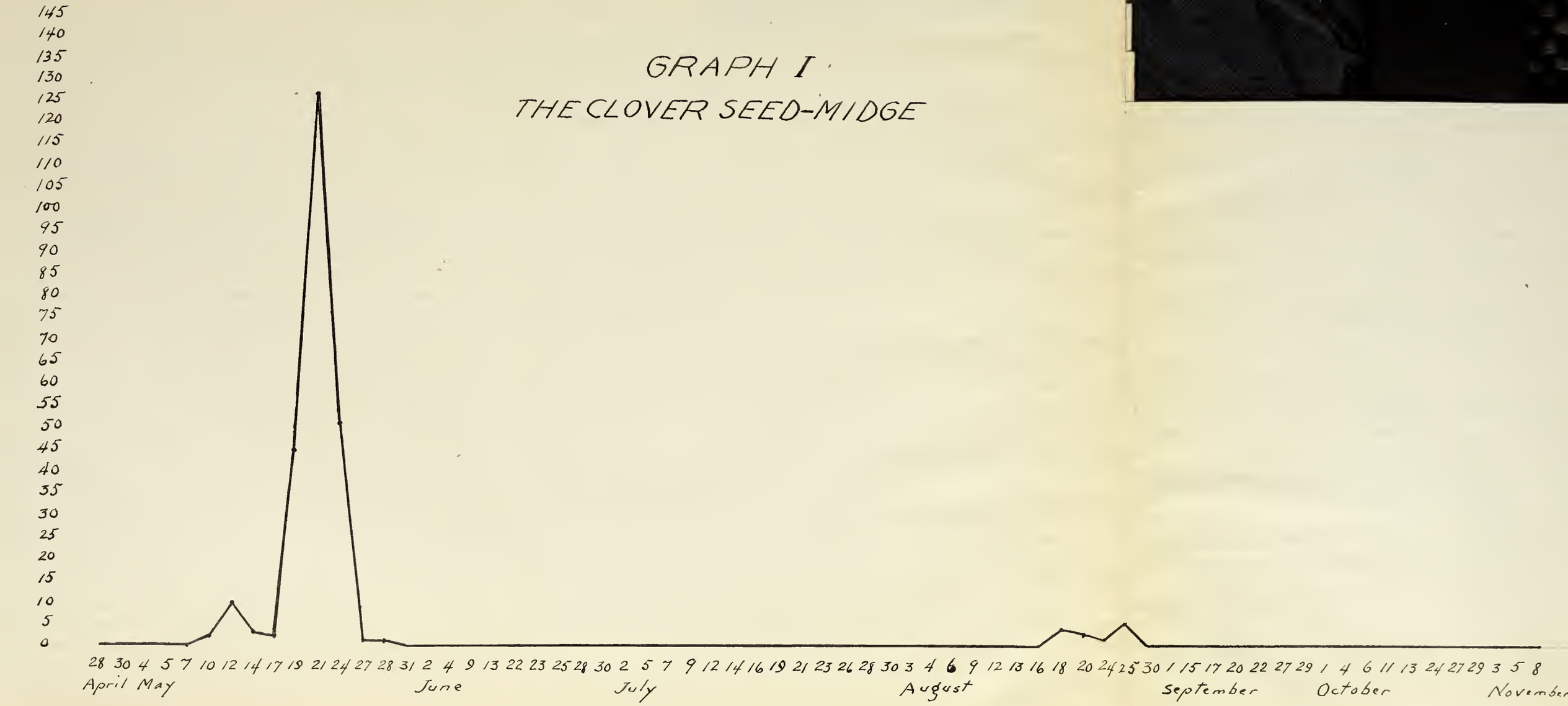
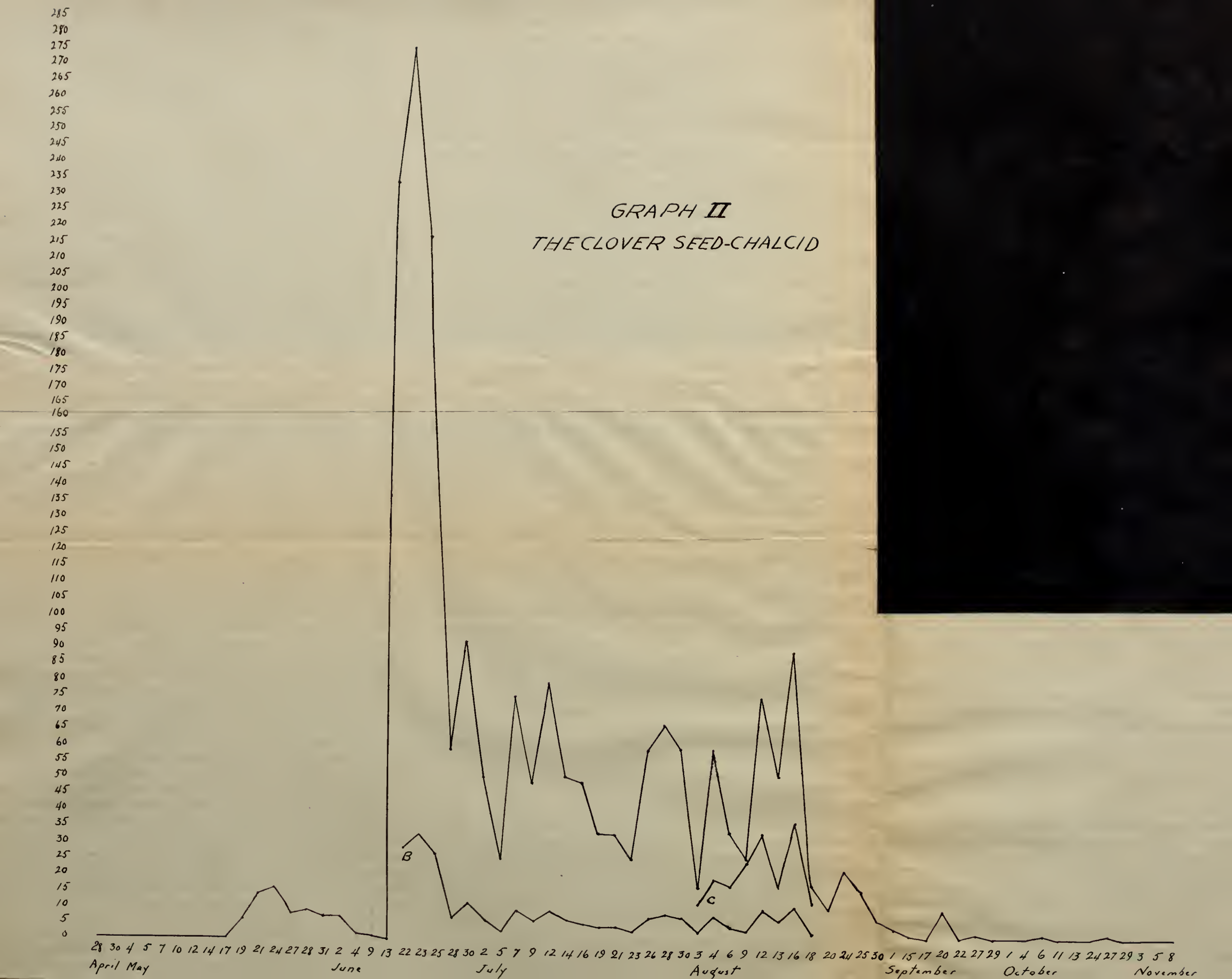
GRAPH I

THE CLOVER SEED-MIDGE



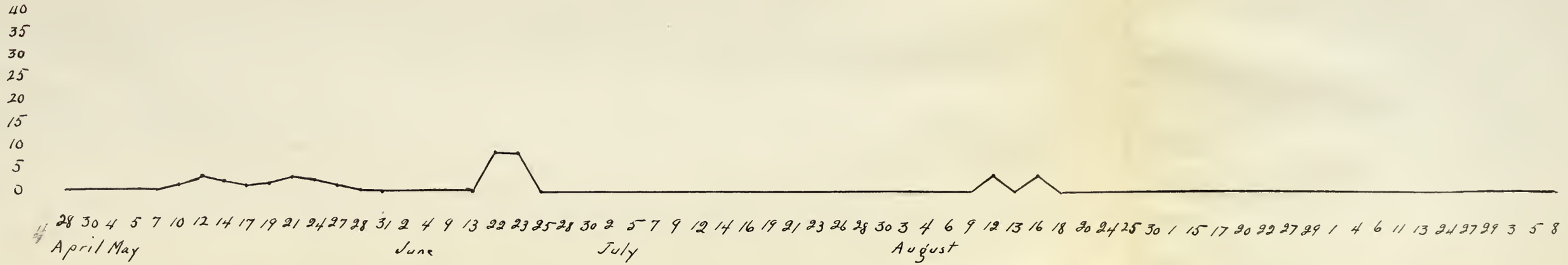
GRAPH II

THE CLOVER SEED-CHALCID



GRAPH III

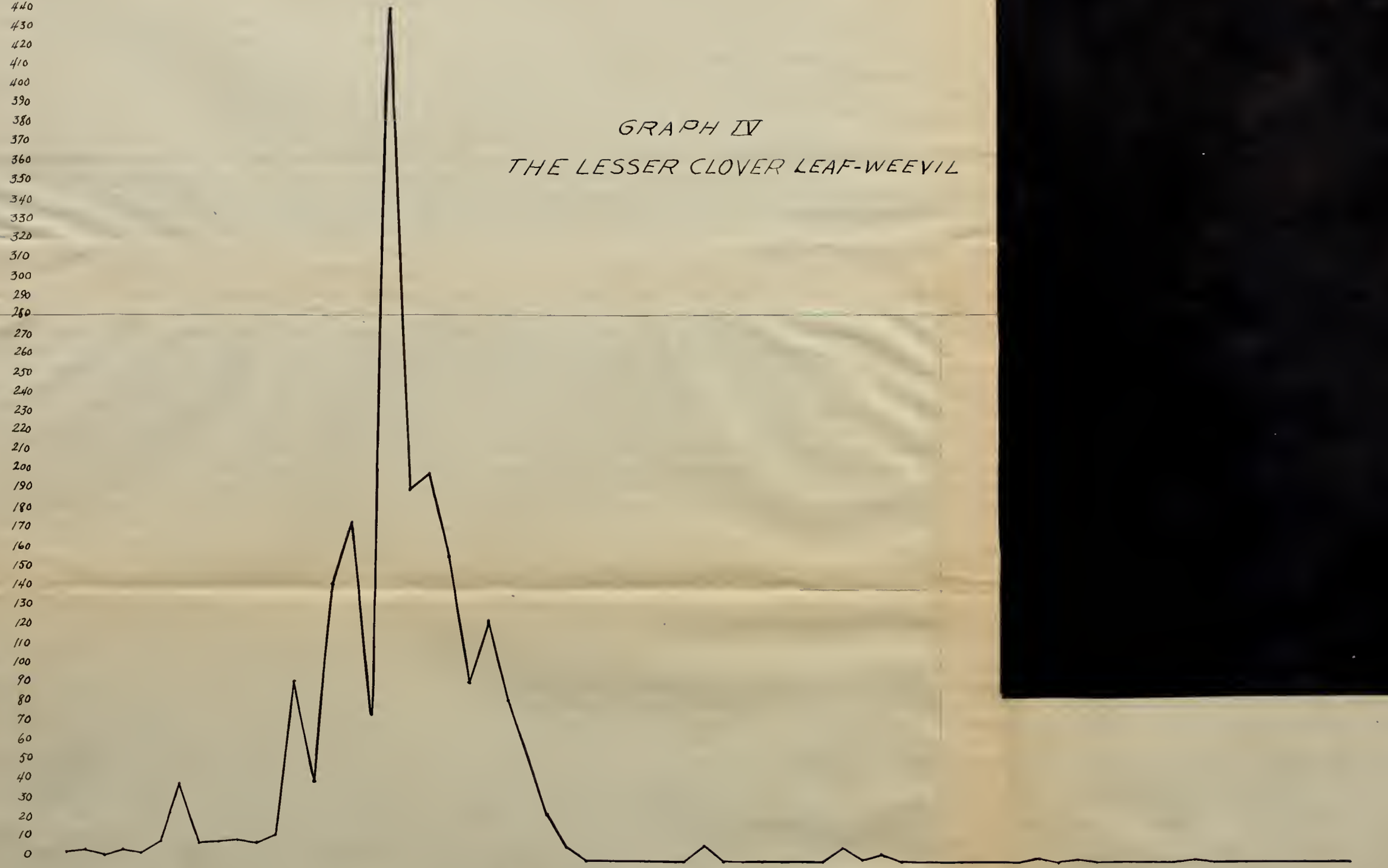
THE CLOVER SEED-CATERPILLAR



GRAPH IV

GRAPH IV

THE LESSER CLOVER LEAF-WEEVIL



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